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Frank C. Eisenschenk

Frank C. Eisenschenk, Ph.D., Patent Attorney

REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 CFR 1.322
Docket No. G-029US04DIV
Patent No. 7,220,722

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Bernard Bihain, Lydie Bougueleret, Frances Yen-Potin
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For	:	Lipoprotein-Regulating Medicaments

Mail Stop Certificate of Corrections Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CERTIFICATE OF CORRECTION
UNDER 37 C.F.R. § 1.322 (OFFICE MISTAKE)

Sir:

A Certificate of Correction (in duplicate) for the above-identified patent has been prepared and is attached hereto.

In the left-hand column below is the column and line number where errors occurred in the patent. In the right-hand column is the page and line number in the application where the correct information appears.

Patent Reads:	**Application Reads:**
Item (54), Title:	Page 1, line 1:
"Lipoprotein-Regulating Medicants"	--Lipoprotein-Regulating Medicaments--
Column 6, line 9:	Page 5, line 33:
"ApM"	--ApM1--

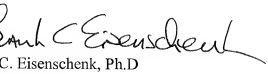
Column 6, line 56:	Page 6, line 22:
"SEQ ID MOs."	--SEQ ID NOs.--
Column 9, line 60:	Page 9, line 22:
"that ran"	--that can--
Column 13, line 47:	Page 13, line 23:
"intratheal"	--intrathecal--
Column 16, line 10:	Page 16, line 6:
"dependent an"	--dependent on--
Column 17, line 10:	Page 17, lines 7-8:
"Acrp segment"	--Acrp 30 segment--
Column 21, line 33:	Page 21, lines 23-24:
"of t coincidence"	--of this coincidence--
Column 23, line 36:	Page 23, line 26:
"bind gC2q-R"	--bind gC1q-R--
Column 24, line 47:	Page 24, line 30:
"Adipose"	--AdipoQ.--
Column 27, line 45:	Page 27, line 29:
"AdivoQ"	--AdipoQ--
Column 34, line 29:	Page 34, line 24:
"(C1q-R)"	--(gC1q-R)--
Column 35, line 33:	Page 35, line 25:
"ribazyme"	--ribozyme--

Column 42, line 67:	Page 43, line 1:
"CAL4"	--GAL4--.

A true and correct copy of pages 1, 5, 6, 9, 13, 16, 17, 21, 23, 24, 27, 34, 35, and 43 of the specification as filed which support Applicants' assertion of the errors on the part of the Patent Office accompanies this Certificate of Correction.

Approval of the Certificate of Correction is respectfully requested.

Respectfully submitted,



Frank C. Eisenschenk, Ph.D
Patent Attorney
Registration No. 45,332
Phone No.: 352-375-8100
Fax No.: 352-372-5800
Address: P.O. Box 142950
Gainesville, FL 32614-2950

FCE/gyl/s1

Attachments: Copy of pages 1, 5, 6, 9, 13, 16, 17, 21, 23, 24, 27, 34, 35, and 43 of the specification

LIPOPROTEIN-REGULATING MEDICAMENTS

Field of the Invention

The present invention relates to medicaments that are useful for modulating lipoprotein levels *in vivo*. More particularly, the invention relates to medicaments that modify the activity of the Lipolysis Stimulated Receptor (LSR) and that can be used to influence the partitioning of dietary lipids between the liver and peripheral tissues, including adipose tissue.

Background of the Invention

Obesity is a public health problem which is both serious and widespread. One-third of the population in industrialized countries has an excess weight of at least 20% relative to the ideal weight. The phenomenon continues to worsen, particularly in regions of the globe where economies are modernizing. In the United States, the number of obese people has escalated from 25% at the end of the 70s to 33% at the beginning of the 90s.

Obesity considerably increases the risk of developing cardiovascular or metabolic diseases. It is estimated that if the entire population had an ideal weight, the risk of coronary insufficiency would decrease by 25% and that of cardiac insufficiency and of cerebral vascular accidents by 35%. Coronary insufficiency, atheromatous disease and cardiac insufficiency are at the forefront of the cardiovascular complications induced by obesity. For an excess weight greater than 30%, the incidence of coronary diseases is doubled in subjects under 50 years. Studies carried out for other diseases are equally eloquent. For an excess weight of 20%, the risk of high blood pressure is doubled. For an excess weight of 30%, the risk of developing a non-insulin-dependent diabetes is tripled. That of hyperlipidemias is multiplied six fold.

The list of diseases having onsets promoted by obesity is long: hyperuricemia (11.4% in obese subjects, against 3.4% in the general population), digestive pathologies, abnormalities in hepatic functions, and even certain cancers.

Whether the physiological changes in obesity are characterized by an increase in the number of adipose cells, or by an increase in the quantity of triglycerides stored in each adipose cell, or by both, this excess weight results mainly from an imbalance between the quantities of calories consumed and those of the calories used by the body. Studies on the causes of this imbalance have been in several directions. Some have focused on studying the mechanism of absorption of foods, and therefore the molecules which control food intake and the feeling of satiety. Other studies have characterized the pathways through which the body uses its calories.

The treatments for obesity which have been proposed are of four types. Food restriction is the most frequently used. The obese individuals are advised to change their dietary habits so as to consume fewer calories. This type of treatment is effective in the short-term. However, the recidivation rate is very high. The increase in calorie use through physical exercise is also proposed. This treatment is ineffective when applied alone, but it improves, however, weight-loss in subjects on a low-calorie diet. Gastrointestinal surgery, which reduces the absorption of the calories ingested, is effective but has been virtually abandoned because of the side effects which it causes. The medicinal approach uses either the anorexigenic action of molecules involved at the level of the central nervous system, or the effect of molecules which increase energy use by increasing the production of heat. The

lower than the initial level. In one embodiment the test animal is a mammal and the method may involve feeding a high-fat meal to this mammal. The high-fat meal can include about 60% fat, about 20% protein, and about 20% carbohydrate. The fat component may include about 37% saturated fatty acids, about 36% polyunsaturated fatty acids and about 36% polyunsaturated fatty acids.

Still another aspect of the invention relates to a method for treating an animal having a condition in which it is desirable to increase the partitioning of dietary lipids to the liver. This method includes the step of administering an LSR agonist to the animal having the condition.

Still yet another aspect of the invention relates to a method for treating an animal having a condition in which it is desirable to decrease the partitioning of dietary lipids to the liver. This method includes the step of administering an LSR antagonist to the animal having the condition.

In another apsect, the invention comprises an agent which increases the activity of a compound which increases the partitioning of dietary lipids to the liver for use as a pharmaceutical. In one embodiment of this aspect, the agent is for use in reducing food intake in obese individuals, reducing the levels of free fatty acids in obese individuals, decreasing the body weight of obese individuals, or treating an obesity related condition selected from the group consisting of atherosclerosis (whether obesity-related or not), obesity-related insulin resistance, obesity-related hypertension, microangiopathic lesions resulting from obesity-related Type II diabetes, ocular lesions caused by microangiopathy in obese subjects with Type II diabetes, and renal lesions caused by microangiopathy in obese subjects with Type II diabetes. In another embodiment of this aspect, the agent increases the activity of adipoQ, ApM1, a compound analogous to adipoQ or ApM1, or the LSR receptor. In a further embodiment of this aspect, the agent is selected from the group consisting of derivatives of adipoQ, ApM1, C1q, derivatives of a compound analogous to any of the preceding compounds wherein the derivatives exhibit greater activity than the corresponding wild type protein and antibodies capable of specifically binding the γ subunit, the C1q receptor (gC1q-R) or a protein related thereto. In yet another embodiment of this apsect the agent is selected from the group consisting of derivatives of compounds comprising at least one of the sequences of SEQ ID NOs.: 1 and 2, derivatives of compounds comprising an amino acid sequence having at least 25% homology to a sequence selected from the group consisting of SEQ ID NOs. 7-14, derivatives of compounds comprising an amino acid sequence having at least 50% homology to a sequence selected from the group consisting of SEQ ID NOs. 7-14, and derivatives of compounds comprising an amino acid sequence having at least 80% homology to a sequence selected from the group consisting of SEQ ID NOs. 7-14, wherein the derivatives exhibit greater activity than the corresponding wild type protein. In still a further embodiment of this aspect, the agent comprises a nucleic acid encoding a polypeptide or protein which influences the partitioning of dietary lipids between the liver and peripheral tissues for use as a medicament. In another embodiment of this aspect, the nucleic acid encodes a protein or polypeptide selected from the group consisting of adipoQ, ApM1, C1q, polypeptides analogous to ApM1, polypeptides having at least one of the consensus sequences of SEQ ID NO: 1 and SEQ ID NO: 2, analogs of any of the preceding polypeptides, homologs of any of the preceding polypeptides, derivatives of any of the preceding polypeptides, and fragments of any of the preceding polypeptides. In still another embodiment of this aspect, the nucleic acid encodes a polypeptide selected

from the group consisting of polypeptides comprising an amino acid sequence having at least 25% homology to one of the sequences of SEQ ID NOs.: 7-14, polypeptides comprising an amino acid sequence having at least 50% homology to one of the sequences of SEQ ID NOs.: 7-14, and polypeptides comprising an amino acid sequence having at least 80% homology to one of the sequences of SEQ ID NOs: 7-14. In a further embodiment of this aspect, the agent is selected from the group consisting of small molecules and drugs. In yet another embodiment of this aspect, the agent is for administration to an individual having a below normal level of activity of adipoQ, ApM1, or an analoguous protein.

Another aspect of the present invention is an agent which decreases the activity of a compound which increases the partitioning of dietary lipids to the liver for use as a pharmaceutical. In one embodiment of this aspect, the agent is for use in treating cachexia in subjects with neoplastic or para-neoplastic syndrome or eating disorders. In another embodiment of this aspect, the agent decreases the activity of adipoQ, ApM1, a compound analogous to adipoQ or ApM1, or the LSR receptor. In a further embodiment of this aspect, the agent is an antibody which binds a compound selected from the group consisting of adipoQ, ApM1, C1q, a protein analogous to any of the preceding proteins, a derivative of adipoQ, C1qa, C1qb, C1qc, mul, cer, ApM1, or acrp which inhibits the activity of wild type adipoQ or wild type ApM1, fragments of any of the preceding polypeptides, the γ subunit, the C1q receptor (gC1q-R) or a protein related thereto. In yet another embodiment of this aspect, the agent is an antibody which binds a polypeptide selected from the group consisting of polypeptides comprising at least one of the sequences of SEQ ID NOs.: 1 and 2, polypeptides comprising an amino acid sequence having at least 25% homology to a sequence selected from the group consisting of SEQ ID NOs. 7-14, polypeptides comprising an amino acid sequence having at least 50% homology to a sequence selected from the group consisting of SEQ ID NOs. 7-14, and polypeptides comprising an amino acid sequence having at least 80% homology to a sequence selected from the group consisting of SEQ ID NOs. 7-14. In a further embodiment of this aspect, the agent is selected from the group consisting of antisense nucleic acids to the adipoQ gene, the ApM1 gene or a portion thereof and nucleic acids capable of forming a triple helix with a portion of the adipoQ gene or the ApM1 gene. In yet another embodiment of this aspect, the agent is selected from the group consisting of antisense nucleic acids to a gene encoding a polypeptide comprising at least one of the sequences of SEQ ID NOs.: 1 and 2, a gene encoding a polypeptide comprising an amino acid sequence having at least 25% homology to a sequence selected from the group consisting of SEQ ID NOs. 7-14, a gene encoeding a polypeptide comprising an amino acid sequence having at least 50% homology to a sequence selected from the group consisting of SEQ ID NOs. 7-14, and a gene encoding a polypeptide comprising an amino acid sequence having at least 80% homology to a sequence selected from the group consisting of SEQ ID NOs. 7-14. In a further embodiment of this aspect, the agent is selected from the group consisting of small molecules and drugs. In a further embodiment of this aspect, the agent is for administration to an individual having a level of adipoQ or ApM1 activity which is above normal.

Another aspect of the present invention is a method for determining whether an obese individual is at risk of suffering from a condition selected from the group consisting of a condition associated with a lower than desirable level of partioning of dietary lipids to the liver, obesity-related atherosclerosis, obesity-related insulin resistance,

Figure 3 shows an alignment of several proteins that are analogous to C1q. The globular domains of proteins belonging to the C1q complement family were aligned using clustalW. The various aligned sequences are:

- C1qa-117: protein sequence of complement C1q A (reference Swiss Prot: P02745), from the amino acid at position 117 (SEQ ID NO:7)
- C1qb-122: protein sequence of complement C1q B (reference Swiss Prot: P02746), from the amino acid at position 122 (SEQ ID NO:8)
- C1qc-121: protein sequence of complement C1q C (reference Swiss Prot: P02747), from the amino acid at position 121 (SEQ ID NO:9)
- mul-1160: protein sequence translated from the nucleic sequence for multimerin (GenBank, Accession: U27109) from amino acid 1160 (SEQ ID NO:14)
- cer-64: protein sequence translated from the nucleic sequence for cerebellin (GenBank, Accession: M58583) from amino acid 64 (SEQ ID NO:10)
- apm1-115: protein sequence translated from the nucleic sequence for ApM1 (GenBank, Accession: D45371) from amino acid 115 (SEQ ID NO:11)
- adQ-118: protein sequence translated from the nucleic sequence for AdipoQ (Genbank, Accession: U49915) from amino acid 118 (SEQ ID NO:12)
- acrp-118: protein sequence translated from nucleic sequence for acrp30 (GenBank, Accession: U37222) from amino acid 118 (SEQ ID NO:13).

Boxed sequences show the two portions of alignment corresponding to the C1q signature, the first corresponding to the consensus deposited in the Prosite data base (#PDOC00857): F-x(5)-[N/D]-x(4)-[F/Y/W/L]-x(6)-F-x(5)-G-x-Y-x-F-x-[F/Y] (SEQ ID NO:1), the second at the COOH-end of the proteins is: [S/T]-x-F-[S/T]-G-[F/Y]-L-[L/V]-[F/Y] (SEQ ID NO:2). In these sequences, the square brackets ([ ]) enclose alternative amino acids that can occupy a position and numbers indicate the number of iterations of an unspecified amino acid. The arrows (V) above the alignments mark the positions of the cystein residues conserved in the three forms of C1q but not in the other aligned proteins. The symbols (*) placed under the alignments indicate the conserved amino acids, the symbols (.) indicate the conservative substitutions of amino acids.

Figures 4A-4C show bar graphs representing different aspects of LSR activity. The graphs show results for (A) binding; (B) uptake or internalization; and (C) degradation of ^{125}I-LDL by cultured hepatocytes. Open bars represent the difference between values obtained after incubation with and without 0.6 mM oleate in the absence of AdipoQ. Closed bars show the same parameters in samples incubated with 25 ng AdipoQ.

Figure 5 is a line graph showing the postprandial lipemic response in rats injected with AdipoQ.

Figures 6A-6B are bar graphs representing results obtained following infusion of AdipoQ in rats. The graphs show results for (A) weight loss; and (B) plasma triglyceride levels.

Figures 7A-7B are bar graphs representing daily food intake for (A) *ob/ob* mice; and (B) *db/db* mice that were either controls or administered with AdipoQ.

While not wishing to be bound by any particular theory of operation, the compounds identified by the methods of the present invention are believed to bind to the γ subunit of the LSR complex whereat the compound either will enhance or inhibit LSR activity. Thus, pharmaceutical compositions comprising a therapeutically effective amount of a compound identified by the process of the invention will be useful for the treatment of diseases characterized by high levels of circulating triglycerides or an undesirably strong tendency for lipid deposition at adipose tissue. Alternatively, compounds that inhibit LSR activity will be useful for favoring lipid deposition to the adipose tissue and/or diminishing liver degradation of dietary lipids.

Thus, in general, the disorders which may be treated with the compounds, compositions, medicaments and pharmaceutical formulations identified by the process of the invention generally refer to disorders involving lipid metabolism.

Pharmaceutical Formulations and Routes of Administration

The identified compounds can be administered to a mammal, including a human patient, alone or in pharmaceutical compositions where they are mixed with suitable carriers or excipient(s) at therapeutically effective doses to treat or ameliorate a variety of disorders associated with lipid metabolism. A therapeutically effective dose further refers to that amount of the compound sufficient to result in amelioration of symptoms as determined by the methods described herein. Thus, a therapeutically effective dosage of AdipoQ or ApM1 will be that dosage of the compound that is adequate to promote reduced triglyceride levels following a high-fat meal and that will promote weight loss with continued periodic use or administration. Techniques for formulation and administration of the compounds of the instant application may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest edition.

Routes of Administration.

Suitable routes of administration include oral, rectal, transmucosal, or intestinal administration, parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal or intraocular injections. A particularly useful method of administering compounds for promoting weight loss involves surgical implantation, for example into the abdominal cavity of the recipient, of a device for delivering the compound over an extended period of time. Sustained release formulations of the invented medicaments particularly are contemplated.

Composition/Formulation

Pharmaceutical compositions and medicaments for use in accordance with the present invention may be formulated in a conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries. Proper formulation is dependent upon the route of administration chosen.

Certain of the medicaments described herein will include a pharmaceutically acceptable carrier and at least one polypeptide that is homologous to the C1q protein or a fragment thereof. In addition to medicaments that include protein components homologous to the C1q protein homologues, we also contemplate that non-protein compounds that interact with the γ subunit of the LSR complex also will find utility as modulators of LSR activity, both *in vitro* and *in vivo*. Included among examples of C1q protein homologues that will find utility in modulating LSR

Dosage intervals can also be determined using the value for the minimum effective concentration. Compounds should be administered using a regimen which maintains plasma levels above the minimum effective concentration for 10-90% of the time, preferably between 30-90%; and most preferably between 50-90%. In cases of local administration or selective uptake, the effective local concentration of the drug may not be related to plasma concentration.

The amount of composition administered will, of course, be dependent on the subject being treated, on the subject's weight, the severity of the affliction, the manner of administration and the judgment of the prescribing physician.

A preferred dosage range for the amount of polypeptide homolog of C1q, such as AdipoQ or ApM1, that can be administered on a daily or regular basis to achieve desired results, including a reduction in levels of circulating plasma triglycerides and/or lipoproteins, range from 0.1 - 50 mg/kg body mass. A more preferred dosage range is from 0.2 - 25 mg/kg. A still more preferred dosage range is from 1.0 - 20 mg/kg, while the most preferred range is from 2.0 - 10 mg/kg. Of course, these daily dosages can be delivered or administered in small amounts periodically during the course of a day.

Protein Homologies

It is to be understood that a polypeptide having a given level of homology to a subject protein or polypeptide can be identified using readily available sequence alignment and comparison programs, such as blastp, fasta and/or ClustalW, and methods that will be familiar to those having ordinary skill in the art. One approach for identifying a protein that is homologous to a subject protein involves running a standard "blastp" (Altschul et al., *J. Mol. Biol.* 215:403-410 (1990) sequence comparison algorithm. In this approach, a relatively low score in the blastp algorithm can be used to isolate large numbers of potentially homologous sequences of different lengths. For example, a low score may be on the order of from between about 80 to about 100 and may result in targets having homology levels of about 20%.

If higher levels of homology are desired, additional steps can be taken. For example, once a first series of candidate homologous sequences has been identified, sequences that are more homologous to the subject protein can then be selected. At that stage, two parameters can be varied. First, it is possible to "cut" the subject protein into sub-sequences of interest and then run homology searches that refine the results obtained in the initial step. For example, the fragments that are listed as SEQ ID 7-14 or the consensus sequences within the two boxed regions shown in Figure 3 could be selected as sub-sequences of interest that could be used to run homology searches. Second, the score used in running the blastp algorithm can be increased. For example, by increasing the score up to a level of about 300, homology levels of about 80% frequently can be obtained. These procedures can be carried out using easily accessible computer programs such as, for example, "blastp" or "fasta". (Altschul et al., *J. Mol. Biol.* 215:403-410 (1990); Pearson, W.R. *Genomics* 11:635-650 (1991)).

Those having ordinary skill in the art will appreciate that the above-referenced score that can be input into the sequence comparison program can be calculated based on the degree of homology that is being sought. Formulas within the cited packages of computer algorithms allow for this possibility. In general, an increasing score allows for

the identification of increasingly specific protein alignments characterized by high levels of homology. Candidate alignments can be further refined using pairwise (fasta) or multiple alignments (ClustalW) (Higgins et al., *Computer Applications in the Biosciences (CABIOS)*, 8:189-191 (1992) and Thompson et al., Nucleic Acids Research 22: 4673-4680 (1994)).

An illustration of the extent of protein homology appears in Table 1. The results appearing in the table show various homology levels between the ApM1 and various whole proteins or fragments of those proteins. Thus, for example, the entire Acrp 30 protein sequence exhibited 81.8% homology with the ApM1 protein, while the Acrp 30 segment identified by SEQ ID NO:13 showed 91.5% homology with the ApM1 protein.

TABLE 1

Homologies Between ApM1 and Various Proteins

Protein/ SEQ ID NO:	Whole protein	Seq ID Nos:7-14
Acrp 30/ SEQ ID NO:13	81.8%	91.5%
AdipoQ/ SEQ ID NO:12	80.6%	90%
C1qa/ SEQ ID NO:7	32.9%	27.1%
C1qb/ SEQ ID NO:8	31.8%	36.7%
C1qc/ SEQ ID NO:9	38.8%	38%
Multimerin/ SEQ ID NO:14	27.7%	28.8%
Cerebellin/ SEQ ID NO:10	24.6%	28.3%

In order to identify a protein having a given level of homology, such as at least 25% homology, at least 50% homology, or at least 80% homology to another protein, such as AdipoQ, ApM1 or C1q, one can use a standard blastp analysis in which the program is instructed to recover proteins having a score corresponding to the desired level of homology. For example, to identify proteins having a homlogy level of 20-30% the program may be instructed to recover proteins having a score from between about 80 to about 100. To recover a protein having an 80% homology level, one can instruct the program to include proteins having a score of about 300. It will be appreciated that these scores may be computed over the full length of the subject protein or over a portion of the protein such as SEQ ID NOs 7-14. It will further be appreciated that homology levels other than those explicitely enumerated herein can be obtained using the instructions provided as part of the program. Thus, the foregoing description is adequate to allow one of ordinary skill in the art to identify polypeptides that are homologous to a subject protein, such as the human ApM1 protein, at various levels of homology. In some cases, the default

LSR antibodies were used to capture membrane proteins from rat liver. After verifying the presence of the 35 kDa species in the column eluate, a sample containing the 35 kDa protein was sequenced using a standard Edman degradation protocol. Results from this procedure gave a 19 amino acid long polypeptide sequence that was used to search a protein data base. This search revealed that the γ subunit of the LSR receptor included a polypeptide sequence that identically appeared in gC1q-R (Ghebrehiwet et al., *J. Exp. Med.* 179:1809 (1994)), a known cell surface receptor that binds the globular heads of C1q. Since the entire sequence of the immunoaffinity purified 35 kDa protein was not established, we allow the possibility that the γ subunit of the LSR complex is related, but not identical to the gC1q-R protein.

Analysis of the protein sequences of the α and β subunits of the LSR revealed several interesting structural features. For example, the presence of several phosphorylation sites at the N-terminal end of the α subunit protein suggested that the amino terminus of this protein was oriented toward the inside of the cell, and further suggested a possible role in signal transduction. The N-terminal portion of the α subunit protein also possessed a hydrophobic amino acid sequence that was separated by two contiguous proline residues, an arrangement likely to induce a hairpin structure. This arrangement of two hydrophobic arms likely constitutes a putative fatty acid binding domain of the LSR. The α subunit also possessed a hydrophobic amino acid sequence consistent with a potential transmembrane domain (Brendel et al., *Proc. Natl. Acad. Sci. USA* 89:2002 (1992)). The β subunit protein does not possess a transmembrane domain and is probably positioned outside of the cell where it is bound through disulfide bridges to other components of the LSR complex.

Compositions and Methods for Modulating LSR Activity

An additional structural feature of the α and β subunit proteins related to the presence of repeated segments that were rich in serine and arginine residues. This was significant because the lamin receptor and "splicing factor 2" also have in common a repeated sequence of serine and arginine residues (RSRS), and these proteins also are known to combine with the gC1qR protein (Honoré et al., *Gene* 134:283 (1993)). In view of this coincidence of related structural motifs and interactions with gC1q-R, we speculated that the serine and arginine rich segments of the LSR α and β subunits were somehow important for contact with gC1q-R, or the gC1q-R-like protein that was the γ subunit of the LSR complex.

As described in the following Example, polyclonal antibodies directed against synthetic peptides derived from the gC1q-R primary amino acid sequence were used to demonstrate that this protein, or a protein closely related to gC1q-R, was a component of the LSR complex. In the procedure described below, the anti-peptide antibodies inhibited the binding of labeled LDL to the LSR expressed on the surface of rat hepatocytes. Use of the LDL model substrate in these procedures provided a convenient and highly sensitive means for monitoring aspects of lipoprotein metabolism in liver cells.

Example 1 describes the procedures used to demonstrate that gC1q-R, or a closely related homologue of this protein, was a constituent of the LSR complex.

Example 2

Regulation of LSR Activity by C1q and its Homologues

Primary cultures of rat hepatocytes were incubated with 20 ng of leptin/well using 6-well plates for 30 minutes at 37°C in order to stimulate mobilization of LSR proteins to the cell surface and to increase the number of LSR receptors expressed. Increasing concentrations of C1q (Sigma) and 20 μg/ml of ^{125}I-LDL were then added to parallel cell cultures in the presence or absence of 0.5 mM oleate. The mixtures were then incubated 4 hours at 37°C and the binding, internalization and degradation of the labeled LDL analyzed using standard techniques (Bihain et al., *Biochemistry* 31:4628 (1992); Mann et al., *J. Biol. Chem.* 272:31348 (1997)).

The results presented in Figures 2A-C unexpectedly indicated that C1q enhanced LSR activity both in the presence and absence of free fatty acids. Indeed, it was surprizing that lipoprotein binding, internalization and degradation occurred in the absence of added oleate because these aspects of LSR activity were previously thought to require the presence of free fatty acids. Small but meaningful increases in all three of the measured parameters also were observed in the presence of oleate. The significance of these latter increases was less substantial because the background values measured in the absence of added C1q were higher in the presence of oleate compared to the values measured in the absence of this free fatty acid.

The results described in the preceeding Example showed that incubation of rat hepatocytes with C1q, a protein capable of binding gC1q-R and hence potentially capable of displacing it from the LSR complex, led to spontaneous activation of the LSR in the absence of free fatty acids. While not wishing to be bound by any particular theory which underlies the mechanism of this receptor modulation, we offer the following as a possible explanation for the phenomenon. It is possible that the gC1q-R protein, or more generally the γ subunit, functions as a chaperon protein for the LSR. It is further possible that the γ subunit somehow exerts an inhibitory effect on the LSR. Conceivably then, agents which perturb or alter the binding of the γ subunit to the LSR can be used to modulate LSR activity which can be measured *in vitro* as the binding, internalization and degradation of LDLs.

The exemplary case presented above suggested that C1q served as the agent that perturbed binding of the γ subunit in the LSR complex. However, we contemplate that any agent homologous or analogous to C1q that is able to bind gC1q-R or a gC1q-R-like protein also will have the effect of modulating LSR receptor activity.

The above-described effect of C1q on the activity of LSR led us to investigate whether similar effects on LSR would be promoted by proteins sharing structural homology with C1q. Alignments for some of these homologues are presented in Figure 3, with the boxed regions representing conserved regions of structural homology. The murine proteins AdipoQ (Hu et al., *J. Biol. Chem.* 271:10697 (1996)) and Acrp30 (Scherer et al., *J. Biol. Chem.* 270:26746 (1995)), and the human ApM1 protein (Maeda et al., *Biochem. Biophys. Res. Commun.* 221:286 (1996)) clearly exhibit marked homologies. These three proteins, like the components of complement C1q (C1q A, B and C), are secreted proteins having N-terminal ends which resemble collagen (repetition of Gly-X-Y motifs), and C-terminal ends corresponding to the globular domain of complement C1q. Significantly, these three proteins are preferentially expressed in adipose tissue. Other protein homologues exhibit globular domains resembling the C1q domain. More specifically, cerebellin and multimerin (isolated in man), are two proteins that do not have a domain which resembles

collagen.

Interestingly, conserved cysteine residues at positions 172, 179, 178 and 190, 196, 192 respectively in C1q A, C1q B and C1q C are not conserved in the other C1q homologues shown in the alignment. These cysteine residues are replaced in ApM1, AdipoQ and Acrp 30, by a lysine residue and an aspartate residue. Those having an ordinary level of skill in the art will appreciate that lysine and aspartate amino acids can, under appropriate conditions, form intrachain salt bridges which may contribute to protein structure. The amino acids at corresponding positions in cerebellin and multimerin would not allow for the formation of salt bridges. It is therefore possible to characterize the C1q domain of the proteins produced by the adipocytes by the absence of cysteines in the region corresponding to amino acids 170-200 of the molecules of C1q and by the consensus in the C1q domain.

When considering the structural relationship of the homologues presented in Figure 3 it is worth noting that the protein ApM1, which is encoded by an mRNA characterized as being strongly expressed in adipocytes, exhibits 79.7% nucleic acid identity and 80.6% amino acid identity with AdipoQ. Given this level of sequence relatedness, the ApM1 protein is almost certainly the human homologue of murine AdipoQ. Thus, it is a reasonable expectation that the activities of murine AdipoQ which are disclosed below also will characterize ApM1 in a human system.

Given that C1q has a broad spectrum of biological effects, including initiation of the complement cascade, it seemed unlikely that the highly specialized activation of the LSR represented a physiologically significant function of this protein. Accordingly, we investigated whether C1q homologues could modulate LSR activity. As indicated in the Examples which follow, we have now demonstrated that AdipoQ, an abundant plasma protein having a heretofore unknown function, also enhances LSR activity.

AdipoQ is a C1q homologue that is known to be secreted by adipocytes with kinetics closely resembling to the kinetics of Adipsin secretion. Adipsin is a hormone of the complement system and has been shown to correspond to the purified fragment of the third component of complement, C3a-desArg (Baldo et al., *J. Clin. Invest.* 92:1543 (1993)). Adipsin stimulates adipocyte triglyceride synthesis and regulates post-prandial lipemia (Sniderman et al., *Proc. Nutr. Soc.* 56:703 (1997)). Moreover, secretion of both AdipoQ and Adipsin is stimulated in response to insulin.

As supported by the experimental results presented below, we have proved that AdipoQ can stimulate LSR activity *in vitro*, and can decrease animal body weight. Since C1q and AdipoQ share structural homology without also sharing extensive functional similarities, our demonstration that AdipoQ activates LSR activity establishes the general utility of C1q homologues as compounds useful for modifying the activity of the LSR.

Example 3 describes the methods used to prepare an expression vector encoding murine AdipoQ.

that had been purchased as a kit (Boehringer Mannheim).

The results presented in Figure 5 show that AdipoQ substantially decreased the magnitude of the postprandial triglyceride response. Quantitative values presented in the Figure represent the mean ± standard deviation (n = 3). Whereas the level of circulating triglycerides remained substantially constant in the animals administered with AdipoQ, the level increased in control animals until reaching a peak at about 4 hours. These *in vivo* results were consistent with the marked AdipoQ-dependent enhancement of LSR activity that we had observed *in vitro*.

Example 7 describes the procedures used to demonstrate that AdipoQ administration promoted weight loss and reduction of plasma triglyceride levels in normal animals. This was true even when the animals were placed on a high-fat diet. Notably, in this case the AdipoQ was administered by a slow infusion protocol instead of by injection.

Example 7

Administration of AdipoQ by Infusion Stimulates Weight Loss and Reduction in Plasma Triglycerides

Osmotic pumps (Alzet) were surgically inserted into the abdominal cavities of 12 male 400-450 g Sprague-Dawley rats. The pumps contained either 2 ml of PBS (pH 7.4) (control, n = 6) or 2 ml mouse recombinant AdipoQ (5 mg/ml PBS, n = 6). The pumps used in this procedure were designed to deliver 10 µl/hour (50 µg AdipoQ/hour). Animals were weighed and then housed individually in metabolic cages. Three animals in each group were put either on regular chow diet or a high-fat diet ad libitum (day 0). The high-fat diet consisted of regular chow supplemented with 2% (w/v) cholesterol, 10% (w/v) saturated fat in the form of vegetaline, 10% (w/v) sunflower oil and 15% (w/v) sucrose. On day 3, the animals were weighed and blood samples were obtained from the tail vein. Plasma triglycerides were measured using an enzymatic kit.

The results presented in Figures 6A-B show that AdipoQ caused a significant reduction in plasma triglyceride levels in test animals fed either a regular or a high fat diet. Moreover, AdipoQ administration caused a reduction in body weight that was more pronounced in animals fed the high fat diet.

Example 8 describes the procedures that defined yet another effect of AdipoQ *in vivo*. More specifically, the results presented below demonstrate that test animals administered with AdipoQ unexpectedly reduced their food intake.

Example 8

AdipoQ Administration Promotes Reduction of Food Intake in Genetically Obese Mice

Both *ob/ob* and *db/db* mice housed in metabolic cages were injected daily for 5 days into the tail veins with either PBS alone or recombinant murine AdipoQ (100 µg) dispersed in a PBS carrier. The amount of food consumed daily by each animal was monitored for the period of the experiment.

The results presented in Figures 7A-B show that the average daily food intake of obese mice was significantly reduced after AdipoQ administration. The graphic data reflect the average food intake and standard deviation for 4 mice in each group, except for the *db/db* control group (n = 3) in which one animal died before the end

aliquots of the dilution placed in wells of a microtiter plate where growth of the culture is continued. Antibody-producing clones are identified by detection of antibody in the supernatant fluid of the wells by immunoassay procedures, such as Elisa, as originally described by Engvall, E., **Meth. Enzymol. 70**:419 (1980), and derivative methods thereof. Selected positive clones can be expanded and their monoclonal antibody product harvested for use. Detailed procedures for monoclonal antibody production are described in Davis, L. et al. **Basic Methods in Molecular Biology** Elsevier, New York. Section 21-2.

Antibodies which are capable of inhibiting the activity of compounds which increase the partitioning of dietary lipids to the liver (including AdipoQ, ApM1, C1q, any of the above-described compounds analogous to C1q, compounds having at least one consensus sequence selected from the group consisting of SEQ ID NO:1 and SEQ ID NO: 2, compounds comprising an amino acid sequence having at least 25% homology to a sequence selected from the group consisting of SEQ ID NOs. 7-14, compounds comprising an amino acid sequence having at least 50% homology to a sequence selected from the group consisting of SEQ ID NOs. 7-14, and compounds comprising an amino acid sequence having at least 80% homology to a sequence selected from the group consisting of SEQ ID NOs. 7-14) may be identified by contacting the compound with increasing amounts of the monoclonal or polyclonal antibodies prior to conducting or while conducting the assay described in Example 5 with the compound. Those antibodies which reduce binding, internalization, and/or degradation in the rat hepatocyte assay may be tested for *in vivo* activity by administering increasing amounts of the antibodies to mice and determining the ability of the antibodies to inhibit the compound-mediated reduction in postprandial triglyceride response in the assay described in Example 6 above, the ability of the antibodies to inhibit the compound-mediated reduction in plasma triglycerides in the assay described in Example 7 above, the ability of the antibodies to inhibit the compound-mediated reduction of food intake in obese mice in the assay described in Example 8 above, or the ability of the antibodies to inhibit the compound-mediated weight loss in the assay described in Example 7.

The partitioning of dietary lipids to the liver may also be reduced by preparing an antibody which binds to the γ subunit, the C1q receptor (gC1q-R) or a protein related thereto, as well as fragments of these proteins. Such antibodies may modulate the interaction between AdipoQ, ApM1, or analogous proteins and the γ subunit, the C1q receptor (gC1q-R) or a protein related thereto in a manner which reduces the partitioning of dietary lipids to the liver. The antibodies may be any of the antibodies described above.

Alternatively, the partitioning of dietary lipids to the liver may also be reduced using fragments of antibodies which retain the ability to specifically bind AdipoQ, ApM1, C1q, any of the above-described compounds analogous to C1q, compounds having at least one consensus sequence selected from the group consisting of SEQ ID NO:1 and SEQ ID NO: 2, compounds comprising an amino acid sequence having at least 25% homology to a sequence selected from the group consisting of SEQ ID NOs. 7-14, compounds comprising an amino acid sequence having at least 50% homology to a sequence selected from the group consisting of SEQ ID NOs. 7-14, compounds comprising an amino acid sequence having at least 80% homology to a sequence selected from the group consisting of SEQ ID NOs. 7-14, the γ subunit, the C1q receptor (gC1q-R) or a protein related thereto, as well as fragments of these proteins. For example, the fragments may be Fab fragments, which may be prepared using methods familiar to those

of skill in the art.

Alternatively, the antibodies may comprise humanized antibodies or single chain antibodies. A variety of methods for making humanized antibodies or single chain antibodies are familiar to those skilled in the art, including the techniques described in U.S. Patent Nos. 5,705,154, 5,565332, and 5,608,039.

Those antibodies which inhibit the compound-mediated effects in one or more of the assays described above may then be used in medicaments for reducing the activity of compounds which increase the partitioning of dietary lipids to the liver. The antibodies may be administered to individuals in a pharmaceutically acceptable carrier such as those described above.

Alternatively, the activity of compounds which increase the partitioning of dietary lipids to the liver (including AdipoQ, ApM1, C1q, any of the above-described compounds analogous to C1q, or compounds having at least one consensus sequence selected from the group consisting of SEQ ID NO:1 and SEQ ID NO: 2, compounds comprising an amino acid sequence having at least 25% homology to a sequence selected from the group consisting of SEQ ID NOs. 7-14, compounds comprising an amino acid sequence having at least 50% homology to a sequence selected from the group consisting of SEQ ID NOs. 7-14, and compounds comprising an amino acid sequence having at least 80% homology to a sequence selected from the group consisting of SEQ ID NOs. 7-14, or a fragment of the preceding compounds) may be reduced by reducing the expression of the genes encoding the compounds. A variety of approaches may be used to reduce gene expression, including antisense or triple helix based strategies.

In antisense approaches, nucleic acid sequences complementary to the mRNA encoding the compound capable of increasing the partitioning of dietary lipids to the liver are hybridized to the mRNA intracellularly, thereby blocking the expression of the protein encoded by the mRNA. The antisense sequences may prevent gene expression through a variety of mechanisms. For example, the antisense sequences may inhibit the ability of ribosomes to translate the mRNA. Alternatively, the antisense sequences may block transport of the mRNA from the nucleus to the cytoplasm, thereby limiting the amount of mRNA available for translation. Another mechanism through which antisense sequences may inhibit gene expression is by interfering with mRNA splicing. In yet another strategy, the antisense nucleic acid may be incorporated in a ribozyme capable of specifically cleaving the target mRNA.

The antisense nucleic acid molecules to be used in gene therapy may be either DNA or RNA sequences. They may comprise a sequence complementary to the sequence of a gene, or a portion of a gene, encoding a compound which increases the partitioning of dietary lipids to the liver. The antisense nucleic acids should have a length and melting temperature sufficient to permit formation of an intracellular duplex having sufficient stability to inhibit the expression of the mRNA in the duplex. Strategies for designing antisense nucleic acids suitable for use in gene therapy are disclosed in Green et al., **Ann. Rev. Biochem.** 55:569-597 (1986) and Izant and Weintraub, **Cell** 36:1007-1015 (1984), which are hereby incorporated by reference.

In some strategies, antisense molecules are obtained from a nucleotide sequence encoding a compound which increases the partitioning of dietary lipids to the liver by reversing the orientation of the coding region with respect to a promoter so as to transcribe the opposite strand from that which is normally transcribed in the cell. The antisense molecules may be transcribed using *in vitro* transcription systems such as those which employ T7 or SP6

domain of GAL4. The two expression plasmids are transformed into yeast and the yeast are plated on selection medium which selects for expression of selectable markers on each of the expression vectors as well as GAL4 dependent expression of the HIS3 gene. Transformants capable of growing on medium lacking histidine are screened for GAL4 dependent lacZ expression. Those cells which are positive in both the histidine selection and the lacZ assay contain plasmids encoding proteins or peptides which interact with the γ subunit, a fragment thereof, or the C1q, AdipoQ or ApM1 binding site.

Alternatively, to study the interaction of the γ subunit, a fragment thereof, or a fragment comprising the C1q, AdipoQ or ApM1 binding site thereof with drugs or small molecules, such as molecules generated through combinatorial chemistry approaches, the microdialysis coupled to HPLC method described by Wang et al., Chromatographia, 44, 205-208(1997) or the affinity capillary electrophoresis method described by Busch et al., J. Chromatogr. 777:311-328 (1997), the disclosures of which are incorporated herein by reference can be used.

In further methods, proteins, peptides, drugs, small molecules, or other compounds which interact with the γ subunit, a fragment thereof, or a fragment comprising the C1q, AdipoQ or ApM1 binding site thereof may be identified using assays such as the following. The molecule to be tested for binding is labeled with a detectable label, such as a fluorescent, radioactive, or enzymatic tag and placed in contact with immobilized γ subunit, a fragment thereof, or a fragment comprising the C1q, AdipoQ or ApM1 binding site thereof under conditions which permit specific binding to occur. After removal of non-specifically bound molecules, bound molecules are detected using appropriate means.

Alternatively, proteins, peptides, drugs, small molecules, or other compounds which bind to γ subunit, a fragment thereof, or a fragment comprising the C1q, AdipoQ or ApM1 binding site thereof may be identified using competition experiments. In such assays, the γ subunit, a fragment thereof, or a fragment comprising the C1q, AdipoQ or ApM1 binding site thereof is immobilized to a surface, such as a plastic plate. Increasing amounts of the proteins, peptides, drugs, small molecules, or other compounds are placed in contact with the immobilized γ subunit, a fragment thereof, or a fragment comprising the C1q, AdipoQ or ApM1 binding site thereof in the presence of a detectably labeled known γ subunit ligand, such as AdipoQ, C1q, any of the above-described compounds analogous to C1q, a compound having at least one consensus sequence selected from the group consisting of SEQ ID NO:1 and SEQ ID NO: 2, a compound comprising an amino acid sequence having at least 25% homology to a sequence selected from the group consisting of SEQ ID NOs. 7-14, a compound comprising an amino acid sequence having at least 50% homology to a sequence selected from the group consisting of SEQ ID NOs. 7-14, or a compound comprising an amino acid sequence having at least 80% homology to a sequence selected from the group consisting of SEQ ID NOs. 7-14. For example, the γ subunit ligand may be detectably labeled with a fluorescent, radioactive, or enzymatic tag. The ability of the test molecule to bind the γ subunit, a fragment thereof, or a fragment comprising the C1q, AdipoQ or ApM1 binding site thereof is determined by measuring the amount of detectably labeled known ligand bound in the presence of the test molecule. A decrease in the amount of known ligand bound to the γ subunit, a fragment thereof, or a fragment comprising the C1q, AdipoQ or ApM1 binding site thereof when the test molecule is present indicates that the test molecule is able to bind to the γ subunit, a fragment thereof, or a fragment comprising the

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,220,722

APPLICATION NO.: 10/072,159

DATED : May 22, 2007

INVENTORS : Bernard Bihain, Lydie Bougueleret, Frances Yen-Potin

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Item (54),
Title, "Lipoprotein-Regulating Medicants" should read
--Lipoprotein-Regulating Medicaments--.

Column 6,
Line 9, "ApM" should read --ApM1--
Line 56, "SEQ ID MOs." should read --SEQ ID NOs.--.

Column 9,
Line 60, "that ran" should read --that can--.

Column 13,
Line 47, "intratheal" should read --intrathecal--.

Column 16,
Line 10, "dependent an" should read --dependent on--.

Column 17,
Line 10, "Acrp segment" should read -- Acrp 30 segment--.

Column 21,
Line 33, "of t coincidence" should read --of this coincidence--.

MAILING ADDRESS OF SENDER:
Saliwanchik, Lloyd & Saliwanchik
P.O. Box 142950
Gainesville, FL 32614-2950

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It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 23,
Line 36, "bind gC2q-R" should read --bind gC1q-R--.

Column 24,
Line 47, "Adipose" should read –AdipoQ.--.

Column 27,
Line 45, "AdivoQ" should read --AdipoQ--.

Column 34,
Line 29, "(C1q-R)" should read --(gC1q-R)--.

Column 35,
Line 33, "ribazyme" should read --ribozyme--.

Column 42,
Line 67, "CAL4" should read --GAL4--.

MAILING ADDRESS OF SENDER:
Saliwanchik, Lloyd & Saliwanchik
P.O. Box 142950
Gainesville, FL 32614-2950